Role of Blimp-1 in programing Th effector cells into IL-10 producers

Christian Neumann,[1,2] Frederik Heinrich,[1] Katrin Neumann,[1,2] Victoria Junghans,[1,2] Mir-Farzin Mashreghi,[1] Jonas Ahlers,[1,2] Marko Janke,[1] Christine Rudolph,[1,2] Nadine Mockel-Tenbrinck,[5] Anja A. Kühl,[3] Markus M. Heimesaat,[4] Charlotte Esser,[6] Sin-Hyeog Im,[7] Andreas Radbruch,[1] Sascha Rutz,[1] and Alexander Scheffold[1,2]

[1]German Rheumatism Research Centre Berlin, an Institute of the Leibniz-Association, 10117 Berlin, Germany
[2]Department of Rheumatology and Clinical Immunology, [3]Medical Clinic I, Gastroenterology, and [4]Department of Microbiology and Hygiene, Charité University Hospital, 10117 Berlin, Germany
[5]Miltenyi Biotec GmbH, 51429 Bergisch Gladbach, Germany
[6]Leibniz Research Institute for Environmental Medicine, 40225 Düsseldorf, Germany
[7]Academy of Immunology and Microbiology (AIM), Institute for Basic Science (IBS) Pohang, Division of Integrative Biosciences and Biotechnology, Pohang University of Science and Technology, Pohang, Republic of Korea

Secretion of the immunosuppressive cytokine interleukin (IL) 10 by effector T cells is an essential mechanism of self-limitation during infection. However, the transcriptional regulation of IL-10 expression in proinflammatory T helper (Th) 1 cells is insufficiently understood. We report a crucial role for the transcriptional regulator Blimp-1, induced by IL-12 in a STAT4-dependent manner, in controlling IL-10 expression in Th1 cells. Blimp-1 deficiency led to excessive inflammation during *Toxoplasma gondii* infection with increased mortality. IL-10 production from Th1 cells was strictly dependent on Blimp-1 but was further enhanced by the synergistic function of c-Maf, a transcriptional regulator of IL-10 induced by multiple factors, such as the Notch pathway. We found Blimp-1 expression, which was also broadly induced by IL-27 in effector T cells, to be antagonized by transforming growth factor (TGF) β. While effectively blocking IL-10 production from Th1 cells, TGF-β shifted IL-10 regulation from a Blimp-1–dependent to a Blimp-1–independent pathway in IL-27–induced Tr1 (T regulatory 1) cells. Our findings further illustrate how IL-10 regulation in Th cells relies on several transcriptional programs that integrate various signals from the environment to fine-tune expression of this critical immunosuppressive cytokine.

CORRESPONDENCE
A. Scheffold:
alexander.scheffold@charite.de
OR
Sascha Rutz:
saschar@gene.com

Abbreviations used: AhR, aryl hydrocarbon receptor; CBA, cytometric bead array; ChIP, chromatin immunoprecipitation; CNS, conserved noncoding sequence; Dll4, Delta-like 4; MARE, Maf recognition element; PFA, paraformaldehyde; qPCR, quantitative PCR; TLA, *Toxoplasma* lysate antigen; Tr1, T regulatory 1; TSS, transcriptional start site.

IL-10, a cytokine with a broad spectrum of anti-inflammatory functions, can suppress immune responses to foreign or self-antigens. During several acute infections, IL-10 is essential to avoid tissue damage as a consequence of excessive inflammation (Moore et al., 2001; Saraiva and O'Garra, 2010; Ouyang et al., 2011). In contrast, various pathogens exploit IL-10 production to evade the immune system leading to chronic infections (Couper et al., 2008). Virtually all cells of the innate and adaptive immune system, including DCs, macrophages, B cells, T helper cells, and cytotoxic T cells, can secrete IL-10 (Saraiva and O'Garra, 2010; Ouyang et al., 2011). However, more recent findings suggest that IL-10 production from effector T cells represents an essential negative feedback mechanism in the self-limitation of inflammatory responses in many infections (Anderson et al., 2007; Jankovic et al., 2007; O'Garra and Vieira, 2007; Sun et al., 2009).

Several factors, including cytokines and cell surface receptors, such as IL-27 (Stumhofer et al., 2007; Anderson et al., 2009; Pot et al., 2009), IL-12 (Chang et al., 2007; Saraiva et al., 2009), TGF-β (Xu et al., 2009), and the Notch pathway (Rutz et al., 2008; Kassner et al., 2010), induce IL-10 production from effector T cells. The corresponding transcriptional programs, however, have only partially been worked out.

S. Rutz and A. Scheffold contributed equally to this paper.
S. Rutz's present address is Genentech, Department of Immunology, South San Francisco, CA 94080

The Rockefeller University Press $30.00
J. Exp. Med. 2014 Vol. 211 No. 9 1807-1819
www.jem.org/cgi/doi/10.1084/jem.20131548

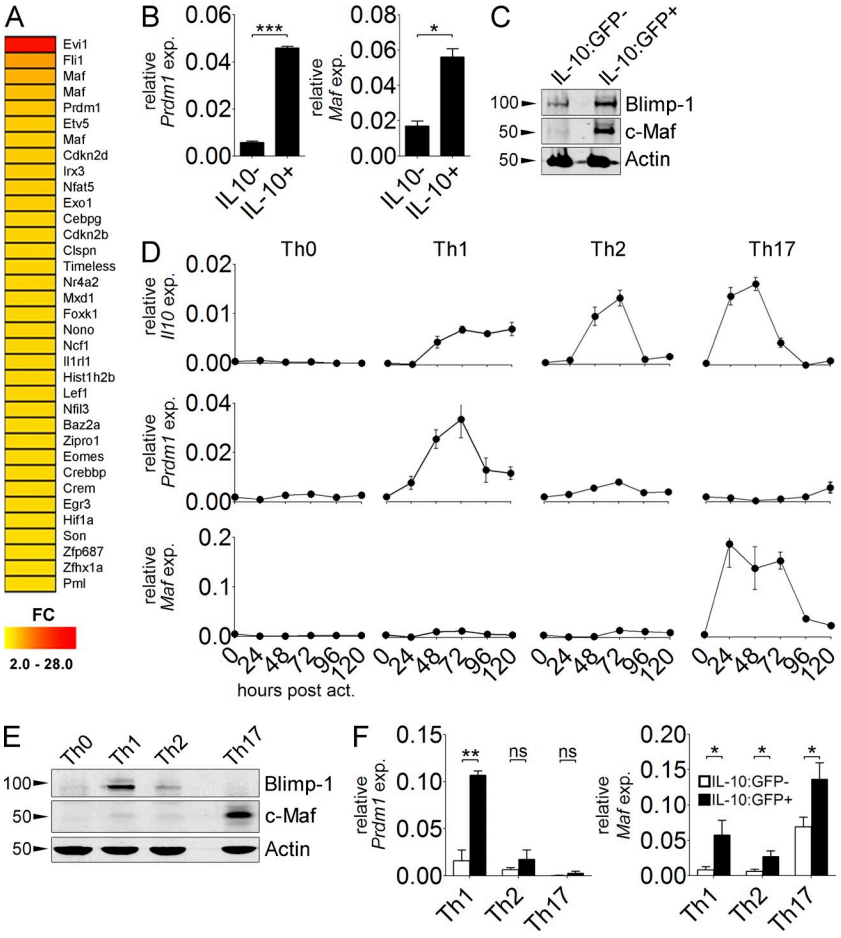

Figure 1. Blimp-1 and c-Maf segregate with IL-10 expression in Th1 cells. (A) Heat map representation of relative expressions of transcription factors >2× up-regulated in in vitro generated IL-10–producing versus nonproducing Th1 cells. Cells were FACS-sorted according to their IL-10 secretion after IL-10 cytokine secretion assay 5 d after activation with anti-CD3/anti-CD28 MACSi Beads under Th1 skewing conditions. Probes were ranked by the difference (log2-fold) between results obtained from IL-10$^+$Th1 cells and IL-10$^-$Th1 cells (FC > 2). (B) Relative *Prdm1* and *Maf* expression in FACS-sorted IL-10–secreting (IL-10$^+$) and nonsecreting (IL-10$^-$) Th1 cells as determined by qPCR. Cells were generated and sorted similarly to A. From left to right: ***, P = 0.0006; *, P = 0.0189. (C) Blimp-1 and c-Maf protein level in IL-10–producing (IL-10:GFP$^+$) and nonproducing (IL-10:GFP$^-$) in vitro generated Th1 cells from IL10:GFP reporter mice 72 h after activation as determined by immunoblot. Indication of protein size is in kD. (D) Relative *Il10*, *Prdm1*, and *Maf* expression time course in indicated in vitro generated Th cell subsets measured by qPCR. (E) Blimp-1 and c-Maf protein expression in indicated in vitro generated Th cell subsets as determined by immunoblot 72 h after T cell priming. Indication of protein size is in kD. (F) Comparison of *Prdm1* and *Maf* expression, measured by qPCR, in IL-10/GFP$^+$ versus IL-10/GFP$^-$ in vitro generated Th1, Th2, and Th17 cells. Cells were FACS-sorted 72 h after starting the Th cell cultures. From left to right: **, P = 0.0089; *, P = 0.037; *, P = 0.048; *, P = 0.043. Data are representative of at least 2 independent experiments (error bars, mean ± SEM).

The transcription factor c-Maf controls IL-10 expression in Th17 and T regulatory 1 (Tr1) cells (Pot et al., 2009; Xu et al., 2009; Apetoh et al., 2010), as well as in macrophages (Cao et al., 2005). c-Maf is induced downstream of IL-27 or TGF-β and binds to consensus motifs (Maf recognition element [MARE]) in the *Il10* promoter. Although c-Maf can transactivate *Il10* by itself to some extent (Xu et al., 2009; Apetoh et al., 2010), robust IL-10 expression seems to require interaction with additional transcriptional regulators. To induce IL-10 in Tr1 cells, c-Maf cooperates with the aryl hydrocarbon receptor (AhR; Apetoh et al., 2010), a ligand-activated transcription factor which is also expressed in Th17 but not in Th1 or Th2 cells. AhR expression is mainly driven by TGF-β (Veldhoen et al., 2008). IL-10 expression from Th2 cells is independent of c-Maf (Kim et al., 1999) but instead requires STAT6 and GATA3 (Chang et al., 2007).

Th1 cells are the major source for IL-10 in many infections, including *Toxoplasma gondii* or *Leishmania major* (Anderson et al., 2007; Jankovic et al., 2007). Yet, the transcriptional regulation of IL-10 in Th1 cells is not well understood. Th1 cells not only lack AhR expression, they also express very low levels of c-Maf (Veldhoen et al., 2008; Pot et al., 2009). IL-12 and the Notch pathway are major drivers of IL-10 production by Th1 cells (Chang et al., 2007; Rutz et al., 2008; Saraiva et al., 2009; Kassner et al., 2010), which is dependent on STAT4 and ERK (Saraiva et al., 2009). In addition, IL-27 is critical for IL-10 production in Th1-driven immune responses in models of infections with *T. gondii* (Stumhofer et al., 2007) or malaria (Freitas do Rosário et al., 2012).

Here, we report that the transcriptional regulator Blimp-1 is critical for IL-10 production in Th1 cells. Blimp-1, which is also involved in IL-10 expression in regulatory T cells as well as in CD8$^+$ cytotoxic T cells (Martins et al., 2006; Cretney et al., 2011), is induced in Th1 cells by IL-12 in a STAT4-dependent manner. We found that Blimp-1–deficient Th1 cells lacked IL-10 production in vitro and in vivo. T cell–specific Blimp-1 deficiency resulted in enhanced inflammation and immunopathology during *T. gondii* infection. c-Maf, although associated with IL-10 in Th1 cells, could not rescue IL-10 expression in the absence of Blimp-1. Both factors bound independently to the *Il10* promoter but acted synergistically to drive IL-10 expression. Under polarizing conditions in vitro, Blimp-1 expression was largely limited to the Th1 subset. However, Blimp-1 was also induced by IL-27 and mediated IL-10 production downstream of IL-27. In contrast, TGF-β, another important driver of IL-10 production, antagonized Blimp-1 while strongly inducing c-Maf. Our data suggest that Blimp-1 is the main driver of IL-10 production in proinflammatory effector T cells downstream of IL-12 and IL-27. In contrast, a c-Maf/AhR–dependent pathway dominates IL-10 regulation in the presence of TGF-β.

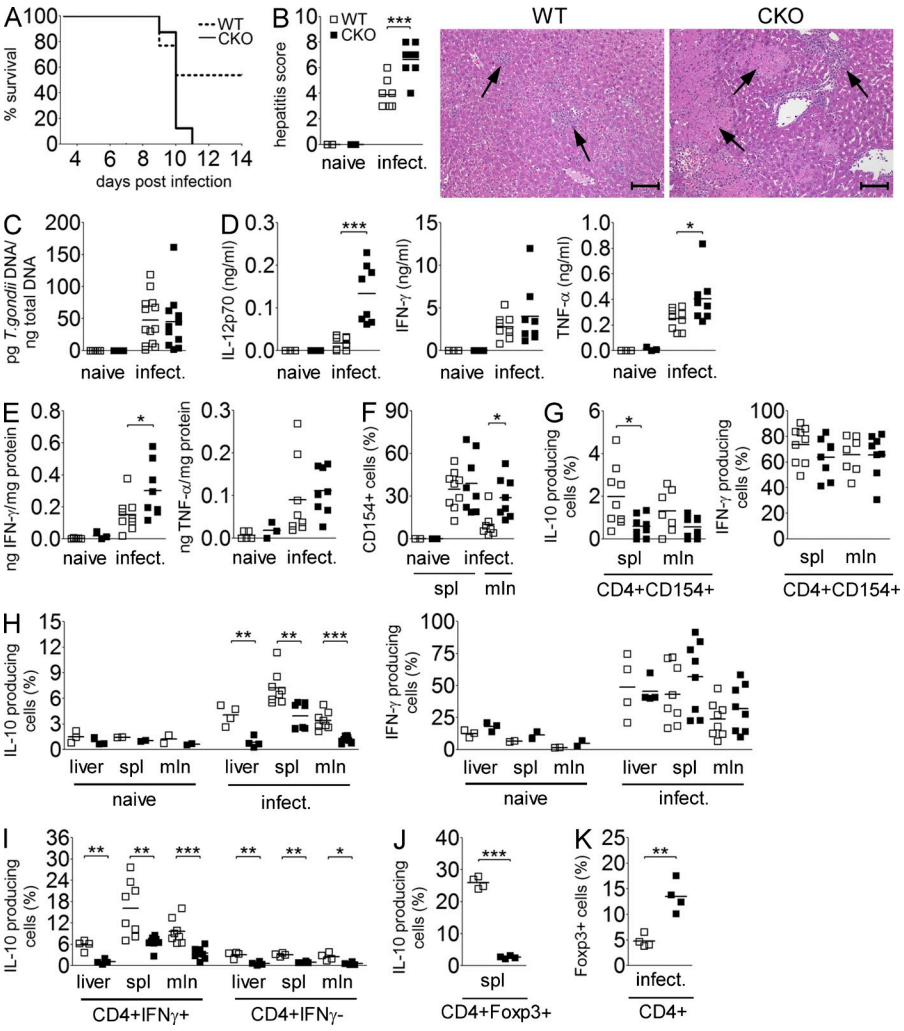

Figure 2. Blimp-1 is critical for limiting Th1-mediated inflammation and for IL-10 production in vivo. Wild-type (*Prdm1*$^{wt/wt}$CD4.Cre; WT) or conditional T cell–specific *Prdm1*-deficient (*Prdm1*$^{fl/fl}$CD4.Cre; CKO) mice were infected with 10 cysts of *T. gondii* or left uninfected (naive). Animals were sacrificed at day 8 after infection for ex vivo analysis. (A) Survival rate of WT and CKO mice after infection. (B) Degree of hepatic inflammation scored by liver histology (left). ***, P = 0.0005. Representative histology staining (H&E staining) of WT and CKO liver sections (right; Bars, 100 µm). Black arrows indicate areas of cell infiltration and necrosis. (C) Abundance of *T. gondii* DNA in ileal biopsies of naive and infected WT or CKO mice. (D) Serum level of IL-12p70, IFN-γ, and TNF as determined by CBA. From left to right: ***, P = 0.0004; *, P = 0.042. (E) Relative amount of IFN-γ and TNF produced during ex vivo overnight liver organ cultures as determined by CBA. *, P = 0.04. (F) Frequency of CD154$^+$ cells among CD4$^+$ cells isolated from spleen (spl) or mesenteric lymph nodes (mln) after ex vivo antigen-specific restimulation with TLA measured by flow cytometry. *, P = 0.01. (G) Frequency of IL-10 and IFN-γ producers among CD4$^+$CD154$^+$ cells after TLA restimulation analyzed by intracellular staining and flow cytometry. *, P = 0.019. (H) Frequency of IL-10$^+$ and IFN-γ$^+$ cells among CD4$^+$ cells isolated from liver, spleen, and mesenteric lymph node was determined by flow cytometry after ex vivo polyclonal restimulation with PMA/ionomycin. From left to right: **, P = 0.0025; **, P = 0.0022; ***, P = 0.0001. (I) Percentage of IL-10–producing cells among CD4$^+$ IFN-γ producing (IFN-γ$^+$) and nonproducing (IFN-γ$^-$) cells after PMA/ionomycin restimulation as determined by flow cytometry. From left to right: **, P = 0.0012; **, P = 0.0042; ***, P = 0.0004; **, P = 0.0027; **, P = 0.0018; *, P = 0.015. (J) Frequency of IL-10$^+$ cells among CD4$^+$Foxp3$^+$ cells isolated from spleen of infected mice after PMA/ionomycin restimulation as determined by flow cytometry. ***, P = 0.001. (K) Frequency of Foxp3$^+$ cells among CD4$^+$ spleen lymphocytes of infected animals measured ex vivo by flow cytometry. **, P = 0.0021. Data are summarized from two (A, B, D–I; *n* = 4/group) or three (C; *n* = 4–5/group) independent experiments, or are representative of two independent experiments (J, K; *n* = 4/group), respectively. Horizontal bars indicate mean.

RESULTS

Transcription factors Blimp-1 and c-Maf are associated with IL-10 expression in Th1 cells

IL-10 production in Th1 cells is driven mainly by IL-12 (Chang et al., 2007; Saraiva et al., 2009). To identify transcription factors that control IL-10 expression in these cells, we generated Th1 cells by culturing naive CD4$^+$ cells with IL-12 for 5 d and performed gene expression profiling of IL-10–secreting Th1 cells after restimulation with PMA/ionomycin. We focused our analysis on transcription factors, which were induced >2× in IL-10-secreting cells (Fig. 1 A). In particular, *Prdm1* (encoding Blimp-1) was expressed at high levels in IL-10–producing Th1 cells compared with IL-10$^-$ Th1 cells as confirmed by quantitative PCR (qPCR; Fig. 1 B) and by Western blot (Fig. 1 C). Similarly, expression of *Maf* (encoding c-Maf) correlated with IL-10 expression in Th1 cells (Fig. 1, B and C). *Evi1* and *Fli*, although identified by gene expression profiling, showed very low overall expression levels and were largely undetectable by qPCR (unpublished data). Blimp-1 had previously been implicated in IL-10 regulation in CD8$^+$ T cells and regulatory T cells (Martins et al., 2006; Cretney et al., 2011), but a respective function in effector T cells has not yet been described. c-Maf, in contrast, controls IL-10 expression in Th17 and Tr1 cells (Pot et al., 2009; Xu et al., 2009; Apetoh et al., 2010) but has not been extensively studied in Th1 cells. To get a better understanding of the expression of both transcription factors in Th1 cells relative to other T effector subsets, we first performed a detailed time-course analysis of Blimp-1 and c-Maf mRNA expression in Th0, Th1, Th2, and Th17 effector T cells (Fig. 1 D). We also compared expression levels by Western blotting (Fig. 1 E) and assessed their association with IL-10 production in the various Th cell subsets (Fig. 1 F). Interestingly, although IL-10

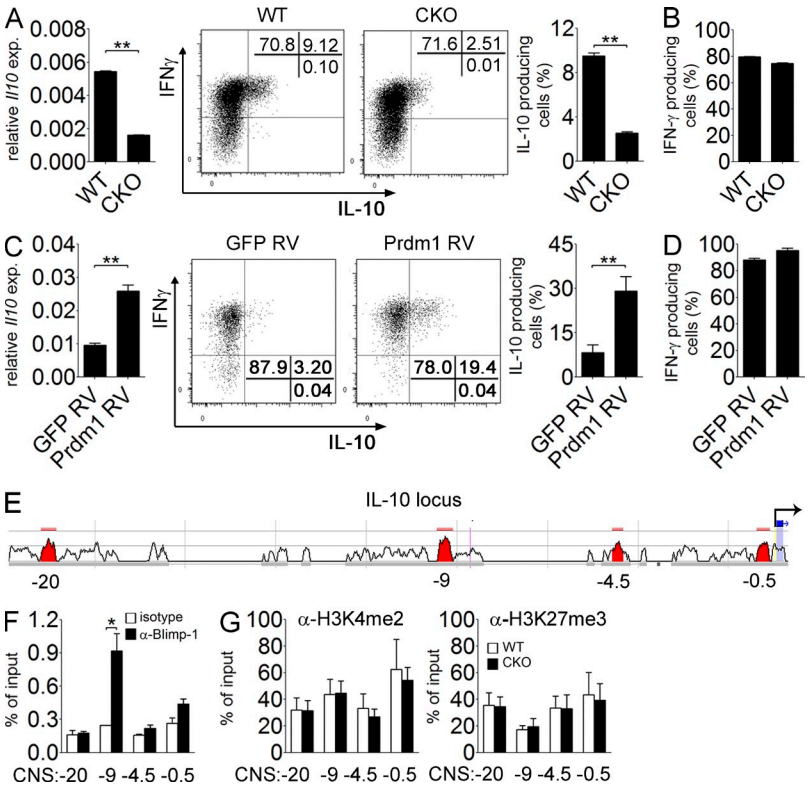

Figure 3. Blimp-1 is crucial for IL-10 expression by Th1 cells in vitro. Naive CD4+ T cells from wild-type (WT) or conditional *Prdm1*-deficient (CKO) mice were cultured in vitro under Th1 skewing conditions. (A and B) Relative *Il10* expression in WT or CKO Th1 cells and frequency of IL-10– (A) and IFN-γ–producing Th1 cells (gated on CD4+; B) 96 h after T cell priming by qPCR or intracellular staining after PMA/ionomycin restimulation, respectively. A representative dot plot is shown. From left to right: **, P = 0.0042; **, P = 0.0031. (C and D) Th1 cells were transduced with either a GFP control (GFP RV) or with a Blimp-1 (Prdm1 RV) retroviral construct. Relative *Il10* expression in GFP-sorted cells and frequency of IL-10 and IFN-γ producers (gated on CD4+GFP+) 96 h after activation after PMA/ionomycin restimulation as determined by qPCR or flow cytometry, respectively. A representative dot plot is shown. From left to right: **, P = 0.0037; **, P = 0.0046. (E) Schematic representation of CNSs in the *Il10* locus upstream of the TSS. Positions of CNS are given in kb relative to TSS. (F) ChIP analysis of Blimp-1 binding to CNS upstream of *Il10* in Th1 cells. Th1 cells were fixed 72 h after the start of culture. *, P = 0.038. (G) ChIP analysis of histone modifications at CNS upstream of *Il10* in WT and CKO Th1 cells 72 h after activation. Data are representative of three (A and B) or two (C and D) independent experiments, or data summarize three (F; duplicates/experiment) or two (G; duplicates/experiment) independent experiments, respectively, (mean ± SEM).

mRNA levels were largely comparable in Th1, Th2, and Th17 cells with some differences in the kinetics of IL-10 expression, Blimp-1 expression was largely confined to Th1 cells (Fig. 1, D and E). We detected only very low levels of Blimp-1 in Th2 cells (Fig. 1, D and E), where it can be induced by IL-4 (Wang et al., 2008). Accordingly, only in Th1 cells, and not in Th2 or Th17 cells, Blimp-1 expression correlated with IL-10 production (Fig. 1 F).

In contrast, c-Maf was predominantly expressed in Th17 but still detectable in Th1 and Th2 cells (Fig. 1, D and E). Although this is in agreement with published data (Bauquet et al., 2009; Pot et al., 2009), it is noteworthy that expression was comparable between Th1 and Th2 cells (Fig. 1, D and E), although originally c-Maf had been described as a Th2 transcription factor (Ho et al., 1996). Interestingly, despite its low level of expression in Th1 and Th2 cells, c-Maf correlated with IL-10 production across all T effector subsets tested (Fig. 1 F). Collectively, its high expression levels and its correlation with IL-10 production selectively in Th1 cells suggested a critical role for Blimp-1 in IL-10 regulation during Th1-mediated immune responses.

Blimp-1 is essential for IL-10 expression by Th1 cells during *T. gondii* infection

IL-10 production from Th1 cells has been reported to be essential to limit immunopathology in various infection models, including *T. gondii* infection (Jankovic et al., 2007). To study the role for Blimp-1 in regulating IL-10 production in Th1-dependent immune responses, we generated T cell–specific conditional *Prdm1*.flox.CD4.cre mice. After peroral infection with 10 cysts of *T. gondii*, *Prdm1*$^{fl/fl}$CD4.Cre mice succumbed to the infection within 11 d, whereas mortality in control mice (*Prdm1*$^{wt/wt}$.CD4.cre) was ∼50% over 14 d (Fig. 2 A). The high susceptibility to infection observed in control mice was probably due to the fact that we used 6–8-wk-old animals to avoid any interference with spontaneous pathology occurring later on in *Prdm1*$^{fl/fl}$CD4.Cre mice (Martins et al., 2006). Consistent with an exacerbated inflammatory response, we observed mild portal lymphocyte infiltration with severe and merging necrosis of the parenchyma in *Prdm1*$^{fl/fl}$CD4.Cre mice, whereas controls showed only focal and minimal to mild infiltration of the liver parenchyma (Fig. 2 B). The parasite burden, however, was similar between the two groups, indicating that parasite control was not compromised in *Prdm1*$^{fl/fl}$CD4.Cre mice (Fig. 2 C). We detected significantly elevated serum levels of IL-12 in *Prdm1*$^{fl/fl}$CD4.Cre mice (Fig. 2 D). TNF and IFN-γ were slightly increased in serum and in the liver, respectively (Fig. 2, D and E). *Prdm1*$^{fl/fl}$CD4.Cre mice exhibited strongly increased frequencies of antigen-specific T cells, particularly in mesenteric lymph nodes (Fig. 2 F). More importantly, IL-10 production was significantly reduced upon antigen-specific restimulation in *Prdm1*-deficient T cells, whereas IFN-γ production was normal (Fig. 2 G). A striking reduction in IL-10 production was observed in CD4+ T cells isolated from liver, spleen, and mesenteric lymph nodes of infected *Prdm1*$^{fl/fl}$CD4.Cre mice as compared with control mice (Fig. 2 H), whereas IFN-γ production from T cells was not altered (Fig. 2 H). Consistent with published data, we found that IL-10 was largely

derived from IFN-γ$^+$ cells in this model (Fig. 2 I). IL-10 production from Foxp3$^+$ regulatory T cells (T reg cells) was also strongly reduced in infected *Prdm1*$^{fl/fl}$CD4.Cre mice (Fig. 2 J), whereas the frequencies of T reg cells were increased (Fig. 2 K) as previously reported (Cretney et al., 2011). Although we cannot exclude a contribution of nonrelated effects of Blimp-1, increased inflammation and elevated serum IL-12 levels were reminiscent of IL-10–deficient mice infected with *T. gondii* (Gazzinelli et al., 1996), suggesting that the reduction in T cell–derived IL-10 in the absence of Blimp-1 contributes to an exacerbated inflammatory response. Collectively, our data supported a critical role for Blimp-1 in regulating IL-10 production in a Th1-driven immune response.

Blimp-1 is required for IL-10 expression from Th1 cells in vitro

Given that Blimp-1 deficiency strongly impaired IL-10 production from Th1 cells in vivo, we wanted to study the role of Blimp-1 in IL-10 regulation in more detail. First, we cultured wild-type and *Prdm1*-deficient T cells under Th1-polarizing conditions in vitro and analyzed IL-10 expression at the mRNA level and after PMA/ionomycin restimulation by intracellular staining. Indeed, both IL-10 mRNA and protein expression in Th1 cells were largely abrogated in the absence of Blimp-1 (Fig. 3 A), whereas the frequency of IFN-γ producers was comparable (Fig. 3 B). Conversely, ectopic expression of Blimp-1 under Th1-polarizing conditions strongly increased IL-10 expression (Fig. 3 C), whereas IFN-γ remained unchanged (Fig. 3 D).

Several conserved noncoding sequences (CNSs) have been identified upstream of the transcriptional start site (TSS) in the *Il10* locus (Fig. 3 E; Fig. S1) and have been implicated in regulating IL-10 expression (Cao et al., 2005; Jones and Flavell, 2005; Chang et al., 2007; Lee et al., 2011). Computational analysis identified putative Blimp-1 binding sites (Fig. S1). Using chromatin immunoprecipitation (ChIP)/qPCR, we detected Blimp-1 binding to a region ∼9 kb upstream of the TSS (CNS-9), but not to other conserved regions at −20, −4.5, or −0.5 kb (Fig. 3 F). We used ChIP/qPCR for Histone H3K4me2 and H3K27me3 in *Prdm1*-deficient versus wild-type Th1 cells to assess whether Blimp-1 binding led to changes in permissive or repressive histone modifications in the *Il10* locus, which might explain the impaired IL-10 expression in the absence of Blimp-1. However, we did not detect any differences in histone H3 methylation in *Prdm1*-deficient Th1 cells (Fig. 3 G). Collectively, these data demonstrated that Blimp-1 was required for IL-10 expression by Th1 cells and suggested that Blimp-1 functioned through direct binding to a regulatory element in the *Il10* locus.

Blimp-1 expression is IL-12/STAT4-dependent in Th1 cells

As previously reported (Chang et al., 2007; Saraiva et al., 2009), IL-10 production in Th1 cells was mainly dependent on IL-12 (Fig. 4 A) and strictly required STAT4 activation, as demonstrated in STAT4-deficient T cells cultured under Th1-polarizing conditions, which lacked IL-10 expression (Fig. 4 B). Because our data suggested that both STAT4 and Blimp-1 were critical, we wanted to better understand the interrelation

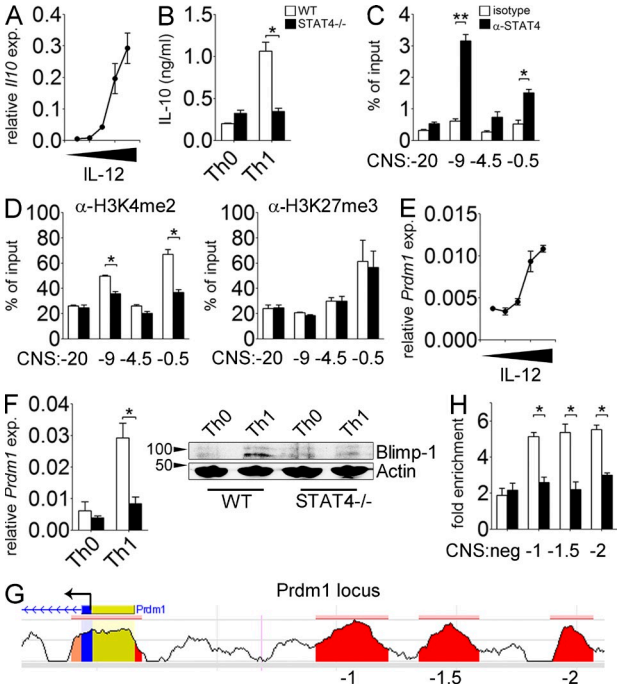

Figure 4. Blimp-1 is strictly IL-12/STAT-4–dependent in Th1 cells. (A) Relative *Il10* expression after exposure of naive Th cells to increasing amounts of rmIL-12 (0, 0.1, 1, 10, and 100 ng/ml) for 48 h as determined by qPCR. (B) Naive CD4$^+$ T cells from wild-type (WT) or STAT4-deficient (STAT4$^{-/-}$) mice were cultured in vitro under Th0 or Th1 skewing conditions, respectively. IL-10 production was measured by ELISA 72 h after Th cell priming. *, P = 0.024. (C) ChIP analysis of STAT4 binding to CNS upstream of *Il10* in Th1 cells 72 h after activation. **, P = 0.0074; *, P = 0.027. (D) ChIP analysis of histone modifications at CNS upstream of *Il10* in WT and STAT4$^{-/-}$ Th1 cells 72 h after activation. From left to right: *, P = 0.019; *, P = 0.021. (E) Relative *Prdm1* expression measured by qPCR after exposure of naive Th cells to increasing amounts of rmIL-12 for 48 h. (F) Relative *Prdm1* expression and Blimp-1 protein level in WT and STAT4$^{-/-}$ Th0 or Th1 cells 72 h after activation as determined by qPCR and immunoblot, respectively. Indication of protein size is in kD. *, P = 0.046. (G) Schematic representation of CNSs upstream of *Prdm1*. Positions of CNS are given in kb relative to TSS. (H) ChIP analysis of STAT4 binding to CNS upstream of *Prdm1* in Th1 cells 72 h after activation. STAT4$^{-/-}$ Th1 cells and a region known to harbor no STAT binding sites (neg) served as negative controls. Data are presented as fold enrichment to isotype control. From left to right: *, P = 0.01; *, P = 0.019; *, P = 0.0062. Data are representative of at least 2 independent experiments (error bars, mean ± SEM).

between these two factors in driving IL-10 production. Using ChIP/qPCR, we found that STAT4 bound in the *Il10* locus the same region (CNS-9) as Blimp-1. In addition, we detected binding further downstream in the CNS-0.5 region (Fig. 4 C; Fig. S1). Interestingly, binding of STAT4 coincided with increased activatory histone H3K4me2 marks in these regions, as demonstrated in comparison with STAT4-deficient T cells cultured under Th1-polarizing conditions (Fig. 4 D). In contrast, repressive H3K27me3 marks were independent of STAT4. These data suggested that STAT4 might regulate *Il10* expression at an epigenetic level by increasing the accessibility of the *Il10* locus.

The high expression of Blimp-1 in Th1 cells but not in other T effector subsets implied that Blimp-1 itself was downstream of IL-12. Indeed, *Prdm1* was induced by IL-12 in a concentration-dependent manner (Fig. 4 E). Moreover, Blimp-1 expression was completely abrogated in the absence of STAT4 in T cells cultured under Th1-polarizing conditions (Fig. 4 F). By ChIP/qPCR, we detected STAT4 binding to conserved STAT binding motifs in CNS regions between 1 and 2 kb upstream of the TSS in the *Prdm1* locus (Fig. 4, G and H; Fig. S2). These data suggested that besides directly binding in the *Il10* locus, STAT4 regulates IL-10 expression also by inducing Blimp-1 expression.

c-Maf acts synergistically with Blimp-1 to induce IL-10 in Th1 cells

Our data clearly demonstrated the critical importance of Blimp-1 for IL-10 expression in Th1 cells. However, our initial gene expression profiling had also revealed a correlation between IL-10 production and expression of c-Maf (Fig. 1). To test whether endogenous c-Maf indeed contributed to IL-10 expression in Th1 cells despite its overall low expression levels, we knocked down c-Maf by RNA interference (Fig. 5 A). Compared with Th1 cells transfected with control siRNA, we detected reduced IL-10 levels by qPCR and ELISA upon siMaf transfection (Fig. 5 B), suggesting that IL-10 expression in Th1 cells was at least partially dependent on c-Maf. Furthermore, ChIP/qPCR analysis revealed c-Maf binding in the CNS-9 region in the *Il10* promoter, the same region bound by Blimp-1 and STAT4 (Fig. 5 C; Fig. S1). c-Maf binding was independent of Blimp-1, as demonstrated by ChIP/qPCR in Blimp-deficient Th1 cells (Fig. 5 D). Moreover, we did not detect a direct interaction between c-Maf and Blimp-1 upon coexpression in HEK 293T cells (unpublished data). Collectively, these data suggested that both factors functioned largely independently of each other. Given the low level expression in Th1 cells (Fig. 1, D and E), we asked whether overexpression of c-Maf would further increase IL-10 production. Indeed, ectopic expression of c-Maf strongly enhanced IL-10 levels while not affecting IFN-γ (Fig. 5 E). We then overexpressed c-Maf in *Prdm1*-deficient Th1 cells to test whether high levels of c-Maf could compensate for Blimp-1 deficiency and restore IL-10. c-Maf by itself only induced moderate IL-10 expression in the absence of Blimp-1 (Fig. 5 E), consistent with previous reports showing that c-Maf can transactivate *Il10* to some extent (Xu et al., 2009; Apetoh et al., 2010). These studies suggested that both factors, although binding independently of each other, functioned synergistically to elicit robust IL-10 production from Th1 cells (Fig. 5, D and E). To directly test the trans-activating capacity of c-Maf and Blimp-1, we cloned the CNS-9 and CNS-4.5 regions of the *Il10* locus in front of a luciferase reporter and performed reporter assays by co-transfecting HEK293 T cells with c-Maf or Blimp-1 or a combination of both. CNS-4.5 served as a control because it was not bound by either factor in Th1 cells. Strikingly, although c-Maf or Blimp-1 alone induced only moderate luciferase activity from the CNS-9 construct, coexpression of c-Maf and Blimp-1 strongly enhanced transcription (Fig. 5 F). Collectively, these data demonstrated that c-Maf could not compensate for Blimp-1 deficiency, and that both factors acted synergistically in driving IL-10 expression.

c-Maf enhances Blimp-1 expression in Th1 cells

Both Blimp-1 and c-Maf expression in Th1 cells was dependent on IL-12 (Fig. 4 E and Fig. 5 G). Given the synergistic effect of both factors in the regulation of IL-10, we asked whether they would also regulate the expression of one another. Therefore, we ectopically expressed c-Maf in T cells under Th0 or Th1 polarizing conditions. Interestingly, although c-Maf was not sufficient to induce Blimp-1 expression in Th0 cells, it further increased Blimp-1 levels in Th1 cells (Fig. 5 H). These data suggested that, although STAT4 downstream of IL-12 is the main driver of Blimp-1 in Th1 cells, c-Maf further enhanced Blimp-1 expression. Consistent with this interpretation, we detected c-Maf binding in the *Prdm1* locus by ChIP/qPCR. Two MARE sites had previously been identified, one in the *Prdm1* promoter (within CNS-2) and another one in intron 5 (CNS+14), the latter one was implicated in the repression of Blimp-1 by Bcl6/Bach2 in B cells (Ochiai et al., 2008; Fig. S2). Interestingly, we found that c-Maf bound to the intron 5 MARE site but not to the promoter (Fig. 5 I). This suggested that c-Maf might enhance Blimp-1 expression by interfering with the repressive function of Bach2, both of which bind to the same DNA motif. In contrast, c-Maf expression was not affected by Blimp-1 (unpublished data).

Notch-mediated IL-10 induction in Th1 cells requires Blimp-1

We had previously reported that activation of the Notch pathway drastically enhances IL-10 production from Th1 cells (Rutz et al., 2008). The Notch-mediated IL-10 induction requires STAT4 activation by IL-12 (Rutz et al., 2008). However, the downstream transcriptional program has not been worked out. Gene expression profiling of Delta-like 4 (Dll4)–stimulated IL-10–secreting Th1 cells suggested that Notch activation did not induce a fundamentally different set of transcription factors (Fig. 6 A and Fig. 1 A). Again, Blimp-1 and c-Maf were strongly expressed in this population as compared with IL-10–negative cells (Fig. 6 A). Therefore, we hypothesized that Notch-induced IL-10 was still dependent on Blimp-1 and c-Maf, and that the much higher IL-10 levels observed upon Notch activation were due to further increased expression of these two factors. Blimp-1 expression in Th1 cells, however, was not significantly up-regulated by Dll4 stimulation, nor was Dll4 sufficient to induce Blimp-1 in Th0 cells (Fig. 6 B). Accordingly, ChIP/qPCR demonstrated comparable Blimp-1 binding in the *Il10* CNS-9 region (Fig. 6 C). In contrast, Dll4 stimulation strongly enhanced c-Maf expression under Th1 polarizing conditions, whereas it was not sufficient to induce c-Maf in Th0 cells (Fig. 6 D). Consistent with its elevated expression in Dll4-stimulated Th1 cells, we detected strongly increased binding of c-Maf to the CNS-9 and CNS-0.5 regions in the *Il10* locus (Fig. 6 E). These observations were reminiscent of our earlier findings upon ectopic expression of c-Maf in Th1 cells (Fig. 5), suggesting that Notch enhanced IL-10 expression

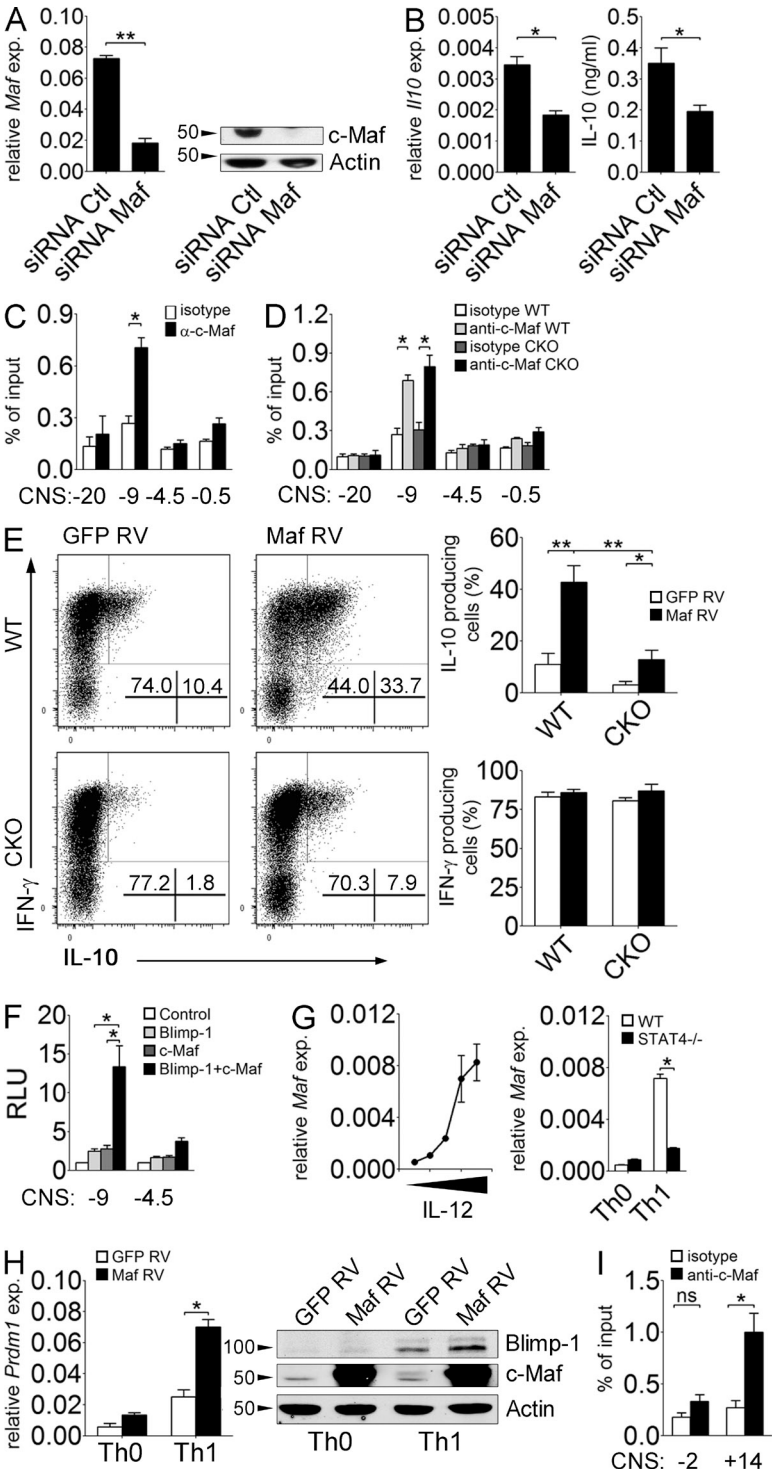

Figure 5. c-Maf acts synergistically with Blimp-1 to induce IL-10 in Th1 cells. (A and B) Naive CD4+ T cells were treated either with a nontargeting control siRNA (siRNA Ctl) or with a c-Maf targeting siRNA (siRNA c-Maf) before Th1 differentiation. (A) Relative *Maf* expression was measured by qPCR for 48 h and c-Maf protein levels were determined by immunoblot 72 h after siRNA treatment. Indication of protein size is in kD. **, P = 0.0021 (B) Relative *Il10* expression determined by qPCR and IL-10 ELISA measured 72 h after the start of Th1 culture and siRNA treatment. From left to right: *, P = 0.017; *, P = 0.032. (C) ChIP analysis of c-Maf binding to CNS upstream of *Il10* in Th1 cells 72 h after activation. *, P = 0.027. (D) ChIP analysis of c-Maf binding to CNS upstream of *Il10* in WT or Prdm1-deficient (CKO) Th1 cells. From left to right: *, P = 0.023; *, P = 0.042. (E) WT or CKO Th1 cells were transduced with either control (GFP RV) or with a c-Maf (Maf RV) retroviral construct. Frequency of IL-10 and IFN-γ producers (gated on CD4+GFP+ cells) was determined by flow cytometry 96 h after priming of Th1 cells after PMA/ionomycin restimulation. A representative dot plot is shown. From left to right: **, P = 0.0025; **, P = 0.007; *, P = 0.021. (F) Luciferase reporter assay in HEK 293T cells transfected with indicated CNS reporter constructs together with empty vector (control), Blimp-1, and/or c-Maf expression plasmids. Firefly luciferase activity was measured 18 h after transfection and is presented relative to constitutive renilla luciferase activity. From left to right: *, P = 0.013; *, P = 0.024. (G) Relative *Maf* expression as determined by qPCR after exposure of naive Th cells to increasing amounts of rmIL-12 (0, 0.1, 1, 10, and 100 ng/ml) for 48 h and in WT or STAT4$^{-/-}$ Th0 and Th1 cells 72 h after priming of naive T cells. *, P = 0.012. (H) Relative *Prdm1* expression and Blimp-1 and c-Maf Protein level in Th0 and Th1 cells after retroviral transduction with GFP RV or c-Maf RV measured by qPCR and immunoblot, respectively. Cells were FACS-sorted for GFP expression 48 h after transduction. Indication of protein size is in kD. *, P = 0.042. (I) ChIP analysis of c-Maf binding to CNS (MARE) sites in the *Prdm1* locus in Th1 cells 72 h after activation. *, P = 0.033. Data are representative of four (A and B) or three (C–I) independent experiments (mean ± SEM).

predominantly by potentiating c-Maf expression and function. Ectopic expression of c-Maf could not compensate for Blimp-1 deficiency to rescue IL-10 expression (Fig. 5); we therefore wanted to test whether Notch-induced IL-10 still required Blimp-1. To address this question, we retrovirally transduced wild-type or Blimp-1–deficient T cells under Th1 polarizing conditions with a constitutively active form of Notch (NICD) or GFP as a control. This represents the strongest possible activation of the Notch pathway and results in dramatic IL-10 induction (Rutz et al., 2008). As expected, NICD strongly induced IL-10 in wild-type T cells (Fig. 6 F). Strikingly, IL-10 production was largely abrogated in NICD-transduced Blimp-1–deficient T cells (Fig. 6 F). However, NICD still induced low levels of IL-10 in the absence of Blimp-1 akin to the effect of ectopic

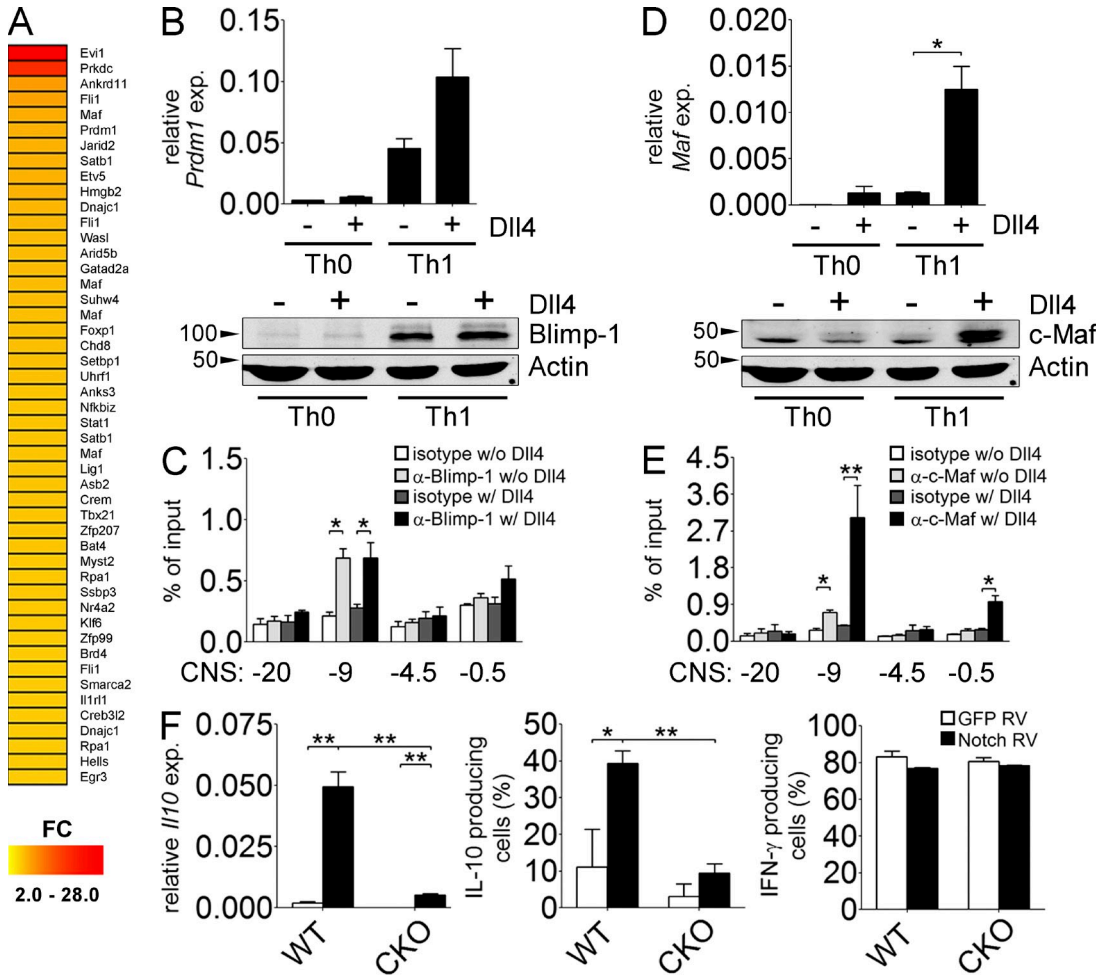

Figure 6. Notch-mediated IL-10 induction in Th1 cells requires Blimp-1. (A) Heat map representation of relative expressions of transcription factors >2× up-regulated in in vitro generated IL-10–producing versus nonproducing Th1 cells stimulated additionally with the Notch ligand Dll4. Cells were FACS-sorted according to their IL-10 secretion after IL-10 cytokine secretion assay 5 d after activation with anti-CD3/anti-CD28 MACSi beads under Th1 skewing conditions. To induce Notch activation, Th1 cells were cultured additionally in the presence of MACSi beads covalently coated with recombinant mouse Dll4. Probes were ranked by the difference (log2-fold) between results obtained from IL-10$^+$Th1 cells and IL-10$^-$Th1 cells (FC > 2). (B) Relative *Prdm1* expression measured by qPCR and Blimp-1 protein level determined by immunoblot in Th0 and Th1 cells stimulated either in the presence (+) or absence (−) of rDll4 72 h after priming of Th cells. Indication of protein size is in kD. (C) ChIP analysis of Blimp-1 binding to CNS upstream of *Il10* in Th1 cells stimulated in the presence (w/) or absence (w/o) of rDll4 72 h after activation. From left to right: *, P = 0.018; *, P = 0.042. (D) Relative *Maf* expression measured by qPCR and c-Maf protein level determined by immunoblot (same blot as in B) in Th0 and Th1 cells stimulated either in the presence (+) or absence (−) of rDll4 72 h after priming of Th cells. Indication of protein size is in kD. *, P = 0.036. (E) ChIP analysis of c-Maf binding to CNS upstream of *Il10* in Th1 cells stimulated in the presence (w/) or absence (w/o) of rDll4 72 h after activation. From left to right: *, P = 0.027; **, P = 0.007; *, P = 0.01. (F) Wild-type (WT) or Prdm1-deficient (CKO) Th1 cells were transduced with either a control (GFP RV) or with a Notch3IC (Notch RV) retroviral construct. Relative *Il10* expression in GFP-sorted cells was measured by qPCR and frequency of IL-10 and IFN-γ producers (gated on CD4$^+$GFP$^+$) was determined by flow cytometry 96 h after Th1 priming after PMA/ionomycin restimulation. From left to right: **, P = 0.002; **, P = 0.003; **, P = 0.002; *, P = 0.038; **, P = 0.009. Data are representative of three independent experiments (mean ± SEM).

c-Maf expression. Collectively, these data demonstrated that, although the Notch-induced IL-10 expression was largely mediated through regulation of c-Maf, overall IL-10 expression from Th1 cells was critically dependent on Blimp-1.

IL-27 induces Blimp-1–dependent IL-10 production in Th cells

Our data clearly demonstrated that IL-12–induced Blimp-1 is instrumental for IL-10 production from Th1 cells. The comparison of Blimp-1 expression among in vitro polarized T effector subsets also suggested that Blimp-1 operated selectively in Th1 cells (Fig. 1, D and E). Accordingly, IL-10 production was not affected in Blimp-1–deficient Th2 and Th17 cells (Fig. 7 A). However, other factors that are typically not represented in polarizing conditions used to differentiate Th cells in vitro are critical for IL-10 production in vivo. One such factor is IL-27, another cytokine of the IL-12 family, which can stimulate IL-10 production from Th0, Th1, Th2, and Tr1 cells (Awasthi et al., 2007; Stumhofer et al., 2007; Anderson et al., 2009; Pot et al.,

2009) and is also critical for IL-10 production from Th1 cells in vivo, for example, during *T. gondii* infection (Stumhofer et al., 2007). The fact that IL-27 induces Blimp-1 expression and Blimp-1–dependent IL-10 production in $CD8^+$ T cells (Sun et al., 2011; Iwasaki et al., 2013) suggested that Blimp-1 might be a more common regulator of IL-10 production in Th cells than it had been reflected in our in vitro cultures. Consistent with this notion, IL-27 induced Blimp-1 expression in naive Th cells. In contrast to stimulation with IL-12, this induction was independent of STAT4 (Fig. 7 B). Similar to our data in Th1 cells, IL-27–induced IL-10 was completely abrogated in *Prdm1*-deficient T cells (Fig. 7 C). These data suggested a much broader role for Blimp-1 in IL-10 regulation in T effector cells.

TGF-β antagonizes Blimp-1–dependent IL-10 production in Th cells

Another factor known to induce IL-10 is TGF-β. In fact, in combination with IL-27, TGF-β further enhances IL-10 production (Awasthi et al., 2007; Stumhofer et al., 2007). Interestingly, although TGF-β induces c-Maf expression (Rutz et al., 2011), a recent report demonstrated that TGF-β suppresses Blimp-1 in Th17 cells (Salehi et al., 2012). We therefore wanted to study the effect of TGF-β on IL-12– and IL-27–induced IL-10 production. Consistent with published data in Th17 cells, TGF-β suppressed Blimp-1 expression in Th1 cells in a concentration-dependent manner. At the same time, it strongly induced c-Maf expression (Fig. 7 D). TGF-β is a potent suppressor of Th1 cells by inhibiting T-bet (Gorelik et al., 2002). Accordingly, TGF-β inhibited IFN-γ production when titrated in Th1 cultures. TGF-β also potently blocked IL-10, further confirming the essential role of Blimp-1 for IL-10 expression in Th1 cells (Fig. 7 E). When combined with IL-27, TGF-β again suppressed Blimp-1 and induced c-Maf expression (Fig. 7 F). However, in contrast to Th1 cells, TGF-β together with IL-27 induced more IL-10 than IL-27 alone (Fig. 7 G), suggesting a transition to Blimp-1–independent pathways of IL-10 expression in the presence of TGF-β. Accordingly, IL-27 only induced IL-10 from *Prdm1*-deficient T cells when TGF-β was present (Fig. 7 H). This was consistent with published data demonstrating that IL-10 in Tr1 cells, which are generated with IL-27 + TGF-β, is driven by c-Maf and AhR (Apetoh et al., 2010). Collectively these data demonstrated that Blimp-1 was critical for IL-10 expression downstream of IL-12 and IL-27, and that TGF-β antagonized this pathway, and instead induced the switch to a Blimp-1–independent and presumably c-Maf/AhR-dependent IL-10 expression as observed in Tr1 and Th17 cells.

DISCUSSION

IL-10 production by effector T cells during inflammatory responses is an important mechanism of self-limitation to avoid the tissue damage that is associated with excessive inflammation

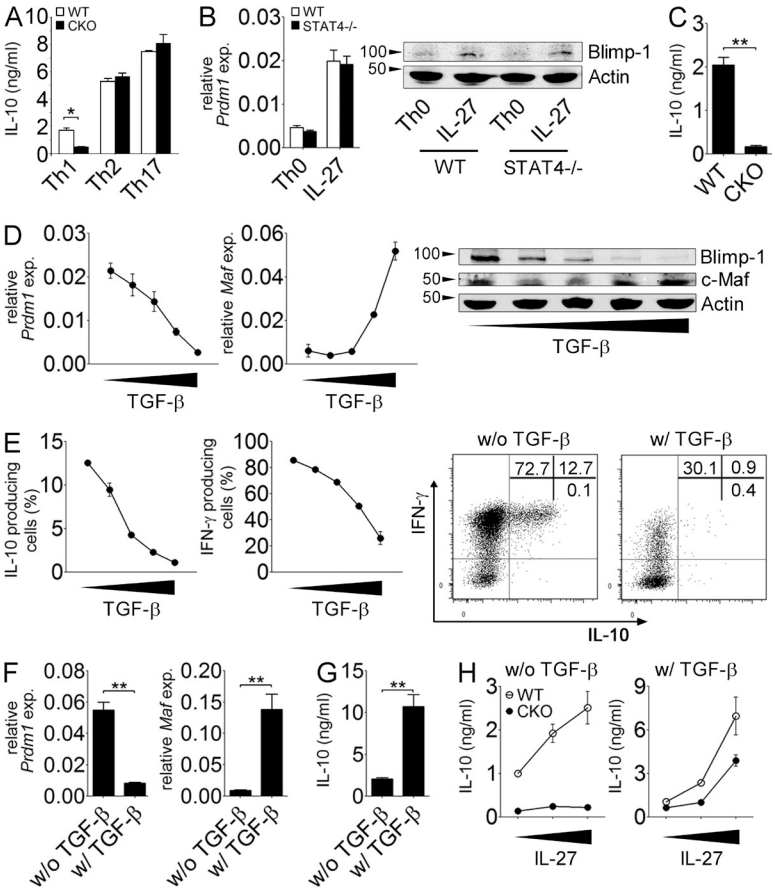

Figure 7. TGF-β antagonizes Blimp-1–dependent IL-10 induction in T cells. (A) Naive $CD4^+$ T cells from wild-type (WT) or conditional *Prdm1*-deficient (CKO) mice were cultured in vitro under Th1, Th2, or Th17 skewing conditions. Production of IL-10 was measured by ELISA 72 h after priming of Th cells. *, P = 0.024. (B) Relative *Prdm1* expression measured by qPCR and Blimp-1 protein levels determined by immunoblot in WT and STAT4-deficient ($STAT4^{-/-}$) T cells upon stimulation with rmIL-27 for 48 h. Indication of protein size is in kD. (C) ELISA of IL-10 produced by WT or CKO T cells stimulated with rmIL-27 for 72 h. **, P = 0.0089. (D) Relative *Prdm1* and *Maf* expression as well as Blimp-1 and c-Maf protein level in Th1 cells cultured in the presence of increasing amounts of rhTGF-β (0, 0.04, 0.2, 1, and 5 ng/ml) for 72 h as determined by qPCR and immunoblot, respectively. Indication of protein size is in kD. (E) Frequency of $IL\text{-}10^+$ and $IFN\text{-}\gamma^+$ T cells cultured under Th1 skewing conditions in the presence of increasing amounts of rhTGF-β was measured by flow cytometry after PMA/ionomycin restimulation 72 h after Th1 priming (gated on $CD4^+$). A representative dot plot of Th1 cells cultured in the absence (w/o) or the presence (w/; 5 ng/ml) of rhTGF-β is shown. (F) Relative *Prdm1* and *Maf* expression in T cells after stimulation with IL-27 (w/o rhTGF-β) or IL-27/rhTGF-β (w/TGF-β; 2 ng/ml) 48 h after activation as determined by qPCR. From left to right: **, P = 0.0011; **, P = 0.009. (G) ELISA of IL-10 produced by IL-27–stimulated T cells in the absence (w/o) or presence (w/) of rhTGF-β (2 ng/ml) measured 72 h after stimulation. **, P = 0.007. (H) IL-10 production by WT or CKO T cells after exposure to increasing amounts of rmIL-27 (1, 5, and 25 ng/ml) in the absence (w/o) or presence (w/; 2ng/ml) of rhTGF-β for 72 h was measured by ELISA. Data are representative of three independent experiments (mean ± SEM).

(O'Garra and Vieira, 2007). Accordingly, effector T cells transiently express IL-10 linked to a state of full activation and effector cytokine production (Saraiva et al., 2009). Although advances have been made to unravel the transcriptional program leading to IL-10 expression in Th17 and Tr1 cells by demonstrating critical functions of c-Maf and AhR (Pot et al., 2009; Xu et al., 2009; Apetoh et al., 2010), the respective mechanisms operating in IFN-γ–producing Th1 cells are less well understood. At least in vitro, Th1-derived IL-10 is known to require IL-12 and STAT4 (Chang et al., 2007; Saraiva et al., 2009). Here, we report that the transcriptional regulator Blimp-1 is induced by IL-12 in a STAT4-dependent manner and is essential for IL-10 production in Th1 cells. Blimp-1, which is best known for its critical function in plasma cell differentiation (Martins and Calame, 2008), is required for IL-10 expression from Th1 cells both in vitro and in vivo. In the absence of Blimp-1, IL-10 production is largely abrogated, whereas IFN-γ production is unaffected. More importantly, mice with a T cell–specific Blimp-1 deficiency show increased inflammation and immunopathology in *T. gondii* infection, suggesting that Blimp-1 significantly contributes to the IL-10–dependent self-limitation of Th1 immune responses.

Blimp-1 expression in Th1 cells in vitro is dependent on STAT4, which explains its late induction during Th1 polarization. The early phase of Th1 differentiation is IL-12 independent and instead relies on IFN-γ–mediated induction of T-bet, which in turn is required for the up-regulation of IL-12Rβ2 (Schulz et al., 2009). Consistent with the transient nature of IL-10 expression in Th1 cells, Blimp-1 activity is restricted to an effector state and is likely to coincide with high availability of proinflammatory cytokines such as IL-12. Indeed, Blimp-1 expression has been found previously to be limited to highly polarized effector $CD4^+$ T cells, and therefore associated with effector cytokine secretion (Martins et al., 2006; Martins and Calame, 2008). The same is true for $CD8^+$ T cells, where Blimp-1 is critical for IL-10 production and its expression is limited to effector and memory $CD8^+$ T cells, and requires CD4 T cell help (Kallies et al., 2006; Martins et al., 2006; Sun et al., 2011). Even in regulatory T cells, Blimp-1 is involved in IL-10 regulation, and is expressed in an effector regulatory T cell population that is found at the site of inflammation (Cretney et al., 2011). Given its expression pattern, Blimp-1 is perfectly suited to limit IL-10 production to an effector phase at the peak of an acute inflammatory response.

In agreement with published studies demonstrating that IL-27 induced Blimp-1 in $CD8^+$ T cells (Sun et al., 2011), we found that IL-27 had the same effect in $CD4^+$ Th cells. IL-27, another cytokine of the IL-12 family, is known to drive IL-10 production in various T cell subsets, including Th1, Th2, and Tr1 cells (Awasthi et al., 2007; Fitzgerald et al., 2007; Stumhofer et al., 2007; Batten et al., 2008). Surprisingly, IL-27–induced IL-10 production in $CD4^+$ T cells was completely dependent on Blimp-1, suggesting a much broader function of Blimp-1 in IL-10 regulation beyond the Th1 subset. In fact, IL-27, although not present in classical polarizing conditions used to differentiate T effector cells in vitro, is critically important for IL-10 production from T cells in vivo, as demonstrated in IL-27R–deficient mice (Awasthi et al., 2007; Stumhofer et al., 2007). Interestingly, during the Th1-driven immune response in *T. gondii* or malaria infection models, IL-27 has been shown to be critical for IL-10 induction in Th1 cells (Stumhofer et al., 2007; Freitas do Rosário et al., 2012), despite the enhanced levels of IL-12 present in these models. Although IL-12 and IL-27 seem to use different pathways to induce Blimp-1, IL-12 by signaling through STAT4 and IL-27 by signaling through STAT1/3 (Stumhofer et al., 2007), their coexpression in many Th1-driven immune responses makes it likely that both cytokines synergize in promoting Blimp-1–dependent IL-10 expression in Th1 cells in vivo.

Interestingly, in Th17 cells (Salehi et al., 2012), and as shown here in Tr1 cells, Blimp-1 expression and function is antagonized by TGF-β. Indeed, we find that ectopic expression of Blimp-1 in Th17 cells strongly induces IL-10 expression (unpublished data). TGF-β, while potently suppressing Blimp-1 expression, induces c-Maf, a transcription factor known to be critical for IL-10 expression in Th17 and Tr1 cells (Pot et al., 2009; Xu et al., 2009; Apetoh et al., 2010; Lee et al., 2012). In fact, for Tr1 cells we demonstrate a TGF-β–induced shift in the regulation of IL-10 expression from a Blimp-1–dependent to a Blimp-1–independent pathway that relies instead on c-Maf/AhR. IL-10 expression in Th17 cells is entirely driven by TGF-β and independent of Blimp-1.

c-Maf expression is induced by a variety of stimuli, including cytokines and cell-based ligands such as IL-27, TGF-β, and ICOS ligand (Bauquet et al., 2009; Pot et al., 2009; Xu et al., 2009; Apetoh et al., 2010), suggesting that c-Maf is part of several transcriptional programs regulating IL-10 expression. Accordingly, we find that Blimp-1 synergizes with c-Maf in Th1 cells to drive IL-10 production. Although c-Maf expression is low in in vitro differentiated Th1 cells compared with other T cell subsets, it can be potently induced by activation of the Notch pathway. We have previously reported that Notch strongly enhances IL-10 production in Th1 cells both in vitro and in vivo when activated through the ligand Dll4 (Rutz et al., 2008; Kassner et al., 2010). Here, we show that Notch activation further up-regulates c-Maf expression and leads to enhanced binding of c-Maf to the *Il10* promoter. Interestingly, although Notch activation did not directly affect Blimp-1 expression or function, Notch-induced IL-10 expression strictly required Blimp-1, demonstrating that Th1 cells most likely cannot use a Blimp-1–independent transcriptional program of IL-10 expression, unlike Tr1 cells.

Our data further demonstrate that distinct transcriptional programs, activated through various signals from the environment, drive IL-10 production in T cells. A better understanding of how T cells integrate these signals into a coherent but highly flexible transcriptional regulation of IL-10 will help to develop more targeted therapeutic strategies.

MATERIALS AND METHODS

Animals. C57BL/6, IL-10:GFP reporter (B6(Cg)-Il10tm1.1Karp/J), and STAT4-deficient mice were purchased from The Jackson Laboratory. *Prdm 1*$^{fl/fl}$ mice (gift from C. Calame, Columbia University, New York, NY) were crossed to CD4.Cre mice (provided by A. Kruglov, German Rheumatism Research Center, Berlin, Germany). All animal experiments were reviewed and approved by the responsible state ethics committee (LAGeSo Berlin, I C 113 – G 0336/08).

T cell purification, activation, and differentiation. $CD4^+CD62L^+CD25^-$ naive T cells were purified by MACS from spleen and lymph nodes. For APC-dependent assays (ectopic overexpression of Blimp-1, c-Maf, and N3ICD), 2.5×10^6 naive T cells were co-cultured with 10^7 MACS-sorted and sublethally irradiated $MHCII^+$ $CD4^-$ cells in 12-well flat-bottom plates in RPMI medium (Gibco). To activate T cells, soluble anti-CD3 (0.5 µg/ml) and anti-CD28 (1 µg/ml) was added (both BD). For APC-free assays, 0.25×10^6 naive T cells were co-cultured with 0.75×10^6 MACSi Beads in 96-well flat-bottom plates. MACSi Beads were coated with anti-CD3 and anti-CD28 (30 µg of total primary IgG antibody per 10^8 beads) before seeding. Notch activation via Dll4 was induced by additional co-culture with MACSi Beads covalently coated with recombinant mouse Dll4. All reagents for APC-free stimulation were purchased from Miltenyi Biotec. To induce T helper cell polarization, the following cytokines and blocking antibodies were added: Th0, 10 ng/ml rmIL-2, 10 µg/ml anti-IL-4 (11B11), 10 µg/ml anti–IFN-γ (AN18.17.24); Th1, 10 ng/ml rmIL-12, 10 ng/ml rmIL-2, 10 µg/ml anti–IL-4; Th2, 10 ng/ml rmIL-4, 10 ng/ml rmIL-2, 10 µg/ml anti–IFN-γ; Th17, 20 ng/ml rmIL-6, 10 ng/ml rmIL-23, 10 ng/ml rmIL-1b, 2 ng/ml rhTGF-β, 10 µg/ml anti–IL-4, 10 µg/ml anti–IFN-γ, and 10 µg/ml anti–IL-2 (S4B6). rmIL-27 was used at a concentration of 20 ng/ml. All polarizing cytokines were purchased from R&D Systems. All blocking antibodies were produced in house.

IL-10 secretion assay. IL-10 secretion assay was performed according to the manufacturer's manual (Miltenyi Biotec). In brief, Th1 polarized cells were restimulated with 10 ng/ml PMA and 1 µg/ml Ionomycin (Sigma-Aldrich) for 3 h at day 5 of in vitro culture. Then, cells were washed to remove any secreted cytokines and labeled with IL-10 Catch Reagent. After a secretion period of 45 min at 37°C, cells were washed again and stained with IL-10 detection antibody (mouse PE–anti–IL-10 AB; Miltenyi Biotec). IL-10 secreting and nonsecreting cells were finally sorted via FACS for further analysis.

Gene array analysis and real-time qPCR. Affymetrix mouse genome 430 2.0 arrays were used to analyze the global gene expression profiles of IL-10–expressing and –nonexpressing Th1 cells (GEO Accession no.: GSE57417). RNA isolation and preparation for the hybridization to the chip was done using the Gene Chip 3′ IVT Express kit. The readout of the signals was performed using the GeneChip Scanner 3000 7G System and the GeneChip Operating Software (GCOS; v1.1.1; Affymetrix). Transcripts up-regulated >2× in IL-10–producing versus nonproducing Th1 cells and a detection p-value <0.05 were analyzed in silico using the AmiGO web interface (Ashburner et al., 2000). Transcripts that were associated with a GO:Term including transcription and/or their involvement in the biological process of positive regulation of gene expression (GO:0010628) were selected. mRNA for real-time PCR was isolated with the RNeasy mini kit according to the manual of the manufacturer (QIAGEN). 100 ng mRNA was used for reverse transcription using the TaqMan Reverse Transcription kit (Applied Biosystems) as it is described in the manufacturer's protocol. qPCR was performed using a LightCycler (Roche) and the LightCycler FastStart DNA Master SYBR Green I kit. The mRNA expression is presented relative to the expression of the housekeeping gene ubiquitin (UBC). Real-time PCR primer can be found in Table S1.

Immunoblot analysis. Total cell protein extracts were resolved on 10% SDS-PAGE gels and transferred to a nitrocellulose membrane. After blocking, membranes were incubated with primary antibodies overnight at 4°C (anti–Blimp-1, 6D3, 1:400 [eBioscience]; anti–c-Maf, M-153X, 1:10,000 [Santa Cruz Biotechnology, Inc.]; anti–β-Actin, C4, 1:10,000 [Santa Cruz Biotechnology, Inc.]). Then, blots were probed with respective IRDye secondary antibody conjugates (LI-COR Biosciences). The final detection was performed using the Odyssey Infrared Imager (LI-COR Biosciences) according to manufacturer's instructions.

***T. gondii*.** Mice were infected perorally with 10 cysts of the *T. gondii* ME49 strain in a volume of 0.3 ml PBS, pH 7.4, by gavage. Cysts were obtained from homogenized brains of NMRI mice that had been infected i.p. with 10 cysts for 2–3 mo. For immunohistochemistry, liver was excised, fixed in 4% neutral buffered formalin, and embedded in paraffin. Thin paraffin sections (1–2 µm) were cut, deparaffinized, and stained with hematoxylin and eosin (H&E). Inflammation was evaluated in H&E-stained section in a blinded manner. The score of hepatitis is the sum of the individual scores for lobular and portal inflammation. Lobular inflammation: 0, no inflammation; 1, minimal inflammation (low inflammatory infiltrate); 2, increased inflammatory cells but less pyknotic necrosis; 3, marked increase in inflammatory cells and lots of pyknotic necrosis; 4, severe inflammation, necrosis; 5, severe inflammation with bridging necrosis. Portal inflammation: 0, uninflamed; 1, mild inflammation (<1/3 of portal tracts); 2, moderate inflammation (approximately 1/2 of portal tracts); 3, severe inflammation (>2/3 of portal tracts); 4, severe portal inflammation dispersing into the parenchyma. Detection of *T. gondii* DNA was performed as previously described (Muñoz et al., 2009). For cytokine detection in liver organ culture supernatants, liver biopsies (5 mm^3) were placed in 24 flat-bottom well culture plates containing 500 µl serum-free RPMI medium supplemented with 100 U/ml penicillin and 100 µg/ml streptomycin (PAA Laboratories). After 18 h at 37°C, culture supernatants were tested for IFN-γ and TNF by cytometric bead array (CBA; BD) and normalized to the amount of total protein in the supernatant. Serum cytokine levels were also measured by CBA at day 8 after infection. For antigen-specific restimulation, ex vivo–derived splenocytes were restimulated with *Toxoplasma* lysate antigen (TLA) for 20 h at 37°C. TLA was generated as previously described (Vossenkämper et al., 2004). After 2 h of stimulation, Brefeldin A (Sigma-Aldrich) was added to block cytokine secretion. To identify antigen-specific T cells, CD154 (Frentsch et al., 2005; Kirchhoff et al., 2007) was labeled with APC–anti-CD154 (Miltenyi Biotec) after the restimulation. Cytokines were stained intracellularly.

Intracellular cytokine staining. In vitro polarized or ex vivo–derived T cells were restimulated with PMA/ionomcyin for 5 h. Brefeldin A was added after 1 h of polyclonal restimulation. Cells were hereafter treated with LIVE/DEAD Fixable Aqua Dead Cell Stain kit (Invitrogen). Then cells were fixed with 2% paraformaldehyde (PFA; Sigma-Aldrich) for 20 min at room temperature. For FACS analysis, cells were stained in 0.5% saponin (Sigma-Aldrich). The following antibodies were used: PacBlue–anti-CD4, PE-Cy7–anti–IFN-γ (both BD), and PE–anti–IL-10 (eBioscience). Foxp3 co-staining was done subsequently to the intracellular cytokine staining using the eBioscience Foxp3 Transcription Factor Staining Buffer Set and according to manufacturer's instructions. FACS analysis was performed on a FACS-Canto (BD).

ELISA. For detection of IL-10 production from in vitro cultures, the IL-10 Ready-SET-Go kit from eBioscience was used according to manufacturer's instructions.

Retroviral transduction. Retroviral supernatants were produced by transfecting HEK 293T cells with pMY-IRES-GFP, pMy-N3ICD-IRES-GFP, pMY-Prdm-1-IRES-GFP, or pMY-c-Maf–IRES-GFP using the calcium phosphate method. Generation and origin of pMY-IRES-GFP and pMY-N3ICD-IRES-GFP, the retroviral packaging plasmid pCGP and pEco was described earlier (Rutz et al., 2008). Prdm1-cDNA (provided by N. Prado, Stanford University, Stanford, CA) was cloned into pMY-IRES-GFP. 24 h after seeding, preactivated T cells from APC–T cell co-cultures were transduced by spin infection (1,800 rpm, 75 min). GFP served as a transfection marker.

ChIP. 72 h after activation, in vitro generated Th1 cells were restimulated for 3 h with PMA/ionomycin and cross-linked first with 2 mM disuccinimidyl glutarate for 45 min and second with 1% PFA for 10 min. PFA fixation was quenched with 125 mM glycin. Cells were subsequently lysed to release chromatin, which was then sheared to a mean size of 300–500 bp by sonication. Blimp-1– (A01647; Genescript), STAT4- (C-20; Santa Cruz Biotechnology, Inc.), or c-Maf (M-153; Santa Cruz Biotechnology, Inc.) – bound DNA sequences were labeled with specific polyclonal antibodies overnight at 4°C. To study chromatin modifications antibodies against dimethyl-histone H4 (Lys4; Millipore) and trimethyl-histone H4 (Lys27; Millipore) were used. Normal rabbit IgG (Cell Signaling Technology) served as a negative control. DNA–protein antibody complexes were immunoprecipitated via μMACS protein A microbeads (Miltenyi Biotec). Cross-linking was reversed by incubation at 65°C for 4 h. ChIP DNA as well as input DNA was purified with DNA extraction kit (MACHEREY-NAGEL). Finally, real-time qPCR was performed to detect the abundance of several CNSs upstream of *Il10* and in the *Prdm1* locus (see Table S2 for primers). Recovered DNA was quantified relative to total input. Analysis of sequence homology and identification of putative regulatory sequences were performed using the ECR browser (http://ecrbrowser.dcode.org) and rVista 2.0 software (Loots et al., 2002).

siRNA. Naive T cells were transfected using the Mouse T Cell Nucleofector kit (Lonza). Nucleofection was performed according to the manufacturer's manual using program X-001 of the nucleofection device. As described earlier (Mantei et al., 2008), chemically modified siRNA was synthesized at IBA as follows (where mC, mU, mA, mT, or mG indicates a methoxynucleotide, and dG, dA, dT, or dC indicates a deoxynucleotide): siRNA specific for cMaf, 5′-mCmUGAUGAAGUUUGAAGUdGdAdAmAmAmA-3′ (sense) or 5′-dTUUCACUUCAAACUUCAUCmAmG-3′ (antisense); and control siRNA, 5′-GmGmAGCGCACCAUCUUCdTdCdAmAmTmT-3′ (sense) or 5′-dTUGAGAAGAUGGUGCGCUmCmC-3′ (antisense).

Luciferase reporter assay. IL-10 reporter plasmids (CNS-9-pXPG and CNS-4.5-pXPG) were generated as previously described (Lee et al., 2011). HEK 293T cells were transfected by calcium phosphate method with IL-10 reporter constructs and the respective overexpression plasmids for Blimp-1 and/or c-Maf. pRL-TK (Promega) served as an internal control plasmid for transfection efficiency. 18 h after transfection, cells were stimulated with PMA/ionomycin for 5 h and luciferase activity was measured by dual luciferase assay system (Promega). Data were normalized to the activity of Renilla luciferase.

Statistics. Data are the mean with SEM and summarize independent experiments as specified in the text. The Student's *t* test (two-sided unpaired) was used to determine the significance of the results.

Online supplemental material. Figs. S1 and S2 show computational analysis of STAT, MARE, and Blimp-1 consensus sequences within CNSs in the *Il10* or *Prdm1* locus, respectively. Tables S1 and S2 list sequences of primer used for qPCR and ChIP-qPCR analysis. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20131548/DC1.

This work was supported by grants from the Deutsche Forschungsgemeinschaft (SFB 633), from the German Federal Ministry of Education and Research (BMBF; 01GU0721), and from the ERC Advanced Grant (ERC-2010-AdG_20100317 grant 268987) to A. Radbruch. M-F. Mashreghi was supported by the "e:Bio – Innovationswettbewerb Systembiologie" program of the Federal Ministry of Education.

We would also like to thank the FCCF, especially Toralf Kaiser and Jenny Kirsch, for flow cytometric cell sorting, Claudia Haftmann and Marina Babic Cac for discussion and reagents, Jannike Sarmadi and Dr. Anja Hauser for providing IL-10 reporter mice, and Dr. Nadine Matzmohr and Dr. Andreas Thiel for providing STAT4-deficient mice.

N. Mockel-Tenbrink is an employee of Miltenyi Biotec. The authors declare no additional competing financial interests.

Submitted: 22 July 2013
Accepted: 27 June 2014

REFERENCES

Anderson, C.F., M. Oukka, V.J. Kuchroo, and D. Sacks. 2007. $CD4^+CD25^-$ $Foxp3^-$ Th1 cells are the source of IL-10-mediated immune suppression in chronic cutaneous leishmaniasis. *J. Exp. Med.* 204:285–297. http://dx.doi.org/10.1084/jem.20061886

Anderson, C.F., J.S. Stumhofer, C.A. Hunter, and D. Sacks. 2009. IL-27 regulates IL-10 and IL-17 from $CD4^+$ cells in nonhealing *Leishmania major* infection. *J. Immunol.* 183:4619–4627. http://dx.doi.org/10.4049/jimmunol.0804024

Apetoh, L., F.J. Quintana, C. Pot, N. Joller, S. Xiao, D. Kumar, E.J. Burns, D.H. Sherr, H.L. Weiner, and V.K. Kuchroo. 2010. The aryl hydrocarbon receptor interacts with c-Maf to promote the differentiation of type 1 regulatory T cells induced by IL-27. *Nat. Immunol.* 11:854–861. http://dx.doi.org/10.1038/ni.1912

Ashburner, M., C.A. Ball, J.A. Blake, D. Botstein, H. Butler, J.M. Cherry, A.P. Davis, K. Dolinski, S.S. Dwight, J.T. Eppig, et al. The Gene Ontology Consortium. 2000. Gene ontology: tool for the unification of biology. *Nat. Genet.* 25:25–29. http://dx.doi.org/10.1038/75556

Awasthi, A., Y. Carrier, J.P. Peron, E. Bettelli, M. Kamanaka, R.A. Flavell, V.K. Kuchroo, M. Oukka, and H.L. Weiner. 2007. A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. *Nat. Immunol.* 8:1380–1389. http://dx.doi.org/10.1038/ni1541

Batten, M., N.M. Kljavin, J. Li, M.J. Walter, F.J. de Sauvage, and N. Ghilardi. 2008. Cutting edge: IL-27 is a potent inducer of IL-10 but not FoxP3 in murine T cells. *J. Immunol.* 180:2752–2756. http://dx.doi.org/10.4049/jimmunol.180.5.2752

Bauquet, A.T., H. Jin, A.M. Paterson, M. Mitsdoerffer, I.-C. Ho, A.H. Sharpe, and V.K. Kuchroo. 2009. The costimulatory molecule ICOS regulates the expression of c-Maf and IL-21 in the development of follicular T helper cells and TH-17 cells. *Nat. Immunol.* 10:167–175. http://dx.doi.org/10.1038/ni.1690

Cao, S., J. Liu, L. Song, and X. Ma. 2005. The protooncogene c-Maf is an essential transcription factor for IL-10 gene expression in macrophages. *J. Immunol.* 174:3484–3492. http://dx.doi.org/10.4049/jimmunol.174.6.3484

Chang, H.D., C. Helbig, L. Tykocinski, S. Kreher, J. Koeck, U. Niesner, and A. Radbruch. 2007. Expression of IL-10 in Th memory lymphocytes is conditional on IL-12 or IL-4, unless the IL-10 gene is imprinted by GATA-3. *Eur. J. Immunol.* 37:807–817. http://dx.doi.org/10.1002/eji.200636385

Couper, K.N., D.G. Blount, and E.M. Riley. 2008. IL-10: the master regulator of immunity to infection. *J. Immunol.* 180:5771–5777. http://dx.doi.org/10.4049/jimmunol.180.9.5771

Cretney, E., A. Xin, W. Shi, M. Minnich, F. Masson, M. Miasari, G.T. Belz, G.K. Smyth, M. Busslinger, S.L. Nutt, and A. Kallies. 2011. The transcription factors Blimp-1 and IRF4 jointly control the differentiation and function of effector regulatory T cells. *Nat. Immunol.* 12:304–311. http://dx.doi.org/10.1038/ni.2006

Fitzgerald, D.C., G.X. Zhang, M. El-Behi, Z. Fonseca-Kelly, H. Li, S. Yu, C.J. Saris, B. Gran, B. Ciric, and A. Rostami. 2007. Suppression of autoimmune inflammation of the central nervous system by interleukin 10 secreted by interleukin 27-stimulated T cells. *Nat. Immunol.* 8:1372–1379. http://dx.doi.org/10.1038/ni1540

Freitas do Rosário, A.P., T. Lamb, P. Spence, R. Stephens, A. Lang, A. Roers, W. Muller, A. O'Garra, and J. Langhorne. 2012. IL-27 promotes IL-10 production by effector Th1 $CD4^+$ T cells: a critical mechanism for protection from severe immunopathology during malaria infection. *J. Immunol.* 188:1178–1190. http://dx.doi.org/10.4049/jimmunol.1102755

Frentsch, M., O. Arbach, D. Kirchhoff, B. Moewes, M. Worm, M. Rothe, A. Scheffold, and A. Thiel. 2005. Direct access to $CD4^+$ T cells specific for defined antigens according to CD154 expression. *Nat. Med.* 11:1118–1124. http://dx.doi.org/10.1038/nm1292

Gazzinelli, R.T., M. Wysocka, S. Hieny, T. Scharton-Kersten, A. Cheever, R. Kühn, W. Müller, G. Trinchieri, and A. Sher. 1996. In the absence of endogenous IL-10, mice acutely infected with *Toxoplasma gondii* succumb to a lethal immune response dependent on CD4+ T cells and accompanied by overproduction of IL-12, IFN-γ and TNF-α. *J. Immunol.* 157:798–805.

Gorelik, L., S. Constant, and R.A. Flavell. 2002. Mechanism of transforming growth factor β-induced inhibition of T helper type 1 differentiation. *J. Exp. Med.* 195:1499–1505. http://dx.doi.org/10.1084/jem.20012076

Ho, I.C., M.R. Hodge, J.W. Rooney, and L.H. Glimcher. 1996. The proto-oncogene c-maf is responsible for tissue-specific expression of interleukin-4. *Cell.* 85:973–983. http://dx.doi.org/10.1016/S0092-8674(00)81299-4

Iwasaki, Y., K. Fujio, T. Okamura, A. Yanai, S. Sumitomo, H. Shoda, T. Tamura, H. Yoshida, P. Charnay, and K. Yamamoto. 2013. Egr-2 transcription factor is required for Blimp-1-mediated IL-10 production in IL-27-stimulated $CD4^+$ T cells. *Eur. J. Immunol.* 43:1063–1073. http://dx.doi.org/10.1002/eji.201242942

Jankovic, D., M.C. Kullberg, C.G. Feng, R.S. Goldszmid, C.M. Collazo, M. Wilson, T.A. Wynn, M. Kamanaka, R.A. Flavell, and A. Sher. 2007. Conventional T-bet($^{(+)}$Foxp3$^{(-)}$) Th1 cells are the major source of host-protective regulatory IL-10 during intracellular protozoan infection. *J. Exp. Med.* 204:273–283. http://dx.doi.org/10.1084/jem.20062175

Jones, E.A., and R.A. Flavell. 2005. Distal enhancer elements transcribe intergenic RNA in the IL-10 family gene cluster. *J. Immunol.* 175:7437–7446. http://dx.doi.org/10.4049/jimmunol.175.11.7437

Kallies, A., E.D. Hawkins, G.T. Belz, D. Metcalf, M. Hommel, L.M. Corcoran, P.D. Hodgkin, and S.L. Nutt. 2006. Transcriptional repressor Blimp-1 is essential for T cell homeostasis and self-tolerance. *Nat. Immunol.* 7:466–474. http://dx.doi.org/10.1038/ni1321

Kassner, N., M. Krueger, H. Yagita, A. Dzionek, A. Hutloff, R. Kroczek, A. Scheffold, and S. Rutz. 2010. Cutting edge: Plasmacytoid dendritic cells induce IL-10 production in T cells via the Delta-like-4/Notch axis. *J. Immunol.* 184:550–554. http://dx.doi.org/10.4049/jimmunol.0903152

Kim, J.I., I.C. Ho, M.J. Grusby, and L.H. Glimcher. 1999. The transcription factor c-Maf controls the production of interleukin-4 but not other Th2 cytokines. *Immunity.* 10:745–751. http://dx.doi.org/10.1016/S1074-7613(00)80073-4

Kirchhoff, D., M. Frentsch, P. Leclerk, D. Bumann, S. Rausch, S. Hartmann, A. Thiel, and A. Scheffold. 2007. Identification and isolation of murine antigen-reactive T cells according to CD154 expression. *Eur. J. Immunol.* 37:2370–2377. http://dx.doi.org/10.1002/eji.200737322

Lee, C.G., W. Hwang, K.E. Maeng, H.K. Kwon, J.S. So, A. Sahoo, S.H. Lee, Z.Y. Park, and S.H. Im. 2011. IRF4 regulates IL-10 gene expression in CD4($^+$) T cells through differential nuclear translocation. *Cell. Immunol.* 268:97–104. http://dx.doi.org/10.1016/j.cellimm.2011.02.008

Lee, Y., A. Awasthi, N. Yosef, F.J. Quintana, S. Xiao, A. Peters, C. Wu, M. Kleinewietfeld, S. Kunder, D.A. Hafler, et al. 2012. Induction and molecular signature of pathogenic TH17 cells. *Nat. Immunol.* 13:991–999. http://dx.doi.org/10.1038/ni.2416

Loots, G.G., I. Ovcharenko, L. Pachter, I. Dubchak, and E.M. Rubin. 2002. rVista for comparative sequence-based discovery of functional transcription factor binding sites. *Genome Res.* 12:832–839. http://dx.doi.org/10.1101/gr.225502.

Mantei, A., S. Rutz, M. Janke, D. Kirchhoff, U. Jung, V. Patzel, U. Vogel, T. Rudel, I. Andreou, M. Weber, and A. Scheffold. 2008. siRNA stabilization prolongs gene knockdown in primary T lymphocytes. *Eur. J. Immunol.* 38:2616–2625. http://dx.doi.org/10.1002/eji.200738075

Martins, G., and K. Calame. 2008. Regulation and functions of Blimp-1 in T and B lymphocytes. *Annu. Rev. Immunol.* 26:133–169. http://dx.doi.org/10.1146/annurev.immunol.26.021607.090241

Martins, G.A., L. Cimmino, M. Shapiro-Shelef, M. Szabolcs, A. Herron, E. Magnusdottir, and K. Calame. 2006. Transcriptional repressor Blimp-1 regulates T cell homeostasis and function. *Nat. Immunol.* 7:457–465. http://dx.doi.org/10.1038/ni1320

Moore, K.W., R. de Waal Malefyt, R.L. Coffman, and A. O'Garra. 2001. Interleukin-10 and the interleukin-10 receptor. *Annu. Rev. Immunol.* 19:683–765. http://dx.doi.org/10.1146/annurev.immunol.19.1.683

Muñoz, M., M.M. Heimesaat, K. Danker, D. Struck, U. Lohmann, R. Plickert, S. Bereswill, A. Fischer, I.R. Dunay, K. Wolk, et al. 2009. Interleukin (IL)-23 mediates *Toxoplasma gondii*-induced immunopathology in the gut via matrixmetalloproteinase-2 and IL-22 but independent of IL-17. *J. Exp. Med.* 206:3047–3059. http://dx.doi.org/10.1084/jem.20090900

Ochiai, K., A. Muto, H. Tanaka, S. Takahashi, and K. Igarashi. 2008. Regulation of the plasma cell transcription factor Blimp-1 gene by Bach2 and Bcl6. *Int. Immunol.* 20:453–460. http://dx.doi.org/10.1093/intimm/dxn005

O'Garra, A., and P. Vieira. 2007. T(H)1 cells control themselves by producing interleukin-10. *Nat. Rev. Immunol.* 7:425–428. http://dx.doi.org/10.1038/nri2097

Ouyang, W., S. Rutz, N.K. Crellin, P.A. Valdez, and S.G. Hymowitz. 2011. Regulation and functions of the IL-10 family of cytokines in inflammation and disease. *Annu. Rev. Immunol.* 29:71–109. http://dx.doi.org/10.1146/annurev-immunol-031210-101312

Pot, C., H. Jin, A. Awasthi, S.M. Liu, C.-Y. Lai, R. Madan, A.H. Sharpe, C.L. Karp, S.-C. Miaw, I.-C. Ho, and V.K. Kuchroo. 2009. Cutting edge: IL-27 induces the transcription factor c-Maf, cytokine IL-21, and the costimulatory receptor ICOS that coordinately act together to promote differentiation of IL-10-producing Tr1 cells. *J. Immunol.* 183:797–801. http://dx.doi.org/10.4049/jimmunol.0901233

Rutz, S., M. Janke, N. Kassner, T. Hohnstein, M. Krueger, and A. Scheffold. 2008. Notch regulates IL-10 production by T helper 1 cells. *Proc. Natl. Acad. Sci. USA.* 105:3497–3502. http://dx.doi.org/10.1073/pnas.0712102105

Rutz, S., R. Noubade, C. Eidenschenk, N. Ota, W. Zeng, Y. Zheng, J. Hackney, J. Ding, H. Singh, and W. Ouyang. 2011. Transcription factor c-Maf mediates the TGF-β-dependent suppression of IL-22 production in T(H)17 cells. *Nat. Immunol.* 12:1238–1245. http://dx.doi.org/10.1038/ni.2134

Salehi, S., R. Bankoti, L. Benevides, J. Willen, M. Couse, J.S. Silva, D. Dhall, E. Meffre, S. Targan, and G.A. Martins. 2012. B lymphocyte-induced maturation protein-1 contributes to intestinal mucosa homeostasis by limiting the number of IL-17-producing $CD4^+$ T cells. *J. Immunol.* 189:5682–5693. http://dx.doi.org/10.4049/jimmunol.1201966

Saraiva, M., and A. O'Garra. 2010. The regulation of IL-10 production by immune cells. *Nat. Rev. Immunol.* 10:170–181. http://dx.doi.org/10.1038/nri2711

Saraiva, M., J.R. Christensen, M. Veldhoen, T.L. Murphy, K.M. Murphy, and A. O'Garra. 2009. Interleukin-10 production by Th1 cells requires interleukin-12-induced STAT4 transcription factor and ERK MAP kinase activation by high antigen dose. *Immunity.* 31:209–219. http://dx.doi.org/10.1016/j.immuni.2009.05.012

Schulz, E.G., L. Mariani, A. Radbruch, and T. Höfer. 2009. Sequential polarization and imprinting of type 1 T helper lymphocytes by interferon-γ and interleukin-12. *Immunity.* 30:673–683. http://dx.doi.org/10.1016/j.immuni.2009.03.013

Stumhofer, J.S., J.S. Silver, A. Laurence, P.M. Porrett, T.H. Harris, L.A. Turka, M. Ernst, C.J. Saris, J.J. O'Shea, and C.A. Hunter. 2007. Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. *Nat. Immunol.* 8:1363–1371. http://dx.doi.org/10.1038/ni1537

Sun, J., R. Madan, C.L. Karp, and T.J. Braciale. 2009. Effector T cells control lung inflammation during acute influenza virus infection by producing IL-10. *Nat. Med.* 15:277–284. http://dx.doi.org/10.1038/nm.1929

Sun, J., H. Dodd, E.K. Moser, R. Sharma, and T.J. Braciale. 2011. $CD4^+$ T cell help and innate-derived IL-27 induce Blimp-1-dependent IL-10 production by antiviral CTLs. *Nat. Immunol.* 12:327–334. http://dx.doi.org/10.1038/ni.1996

Veldhoen, M., K. Hirota, A.M. Westendorf, J. Buer, L. Dumoutier, J.-C. Renauld, and B. Stockinger. 2008. The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. *Nature.* 453:106–109. http://dx.doi.org/10.1038/nature06881

Vossenkämper, A., D. Struck, C. Alvarado-Esquivel, T. Went, K. Takeda, S. Akira, K. Pfeffer, G. Alber, M. Lochner, I. Förster, and O. Liesenfeld. 2004. Both IL-12 and IL-18 contribute to small intestinal Th1-type immunopathology following oral infection with Toxoplasma gondii, but IL-12 is dominant over IL-18 in parasite control. *Eur. J. Immunol.* 34:3197–3207. http://dx.doi.org/10.1002/eji.200424993

Wang, L., N. van Panhuys, J. Hu-Li, S. Kim, G. Le Gros, and B. Min. 2008. Blimp-1 induced by IL-4 plays a critical role in suppressing IL-2 production in activated CD4 T cells. *J. Immunol.* 181:5249–5256. http://dx.doi.org/10.4049/jimmunol.181.8.5249

Xu, J., Y. Yang, G. Qiu, G. Lal, Z. Wu, D.E. Levy, J.C. Ochando, J.S. Bromberg, and Y. Ding. 2009. c-Maf regulates IL-10 expression during Th17 polarization. *J. Immunol.* 182:6226–6236. http://dx.doi.org/10.4049/jimmunol.0900123